Isolation, Identification and Evaluation of Seed Germination Efficiency of Cyanobacterial Isolates

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Abstract: **- Cyanobacteria are diverse group of oxygenic photosynthetic prokaryotes. Cyanobacteria produces many metabolites including amino acids, protein, vitamins, siderophores and plant growth regulators i.g. IAA (Indole 3-acetic acid). A screening of eight cyanobacterial strains in logarithmic and late logarithmic phase was carried out to study the effect of cell free supernatant on wheat and mung seed germination and growth. The strains showed 100% germination in case of wheat and mung respectively, after three days of inoculation as compared to only 70% germination in control. There was variable response in term of root growth, and fresh weight of seedlings of both mung and wheat in presence of cell free supernatant.**

Key Words**: Cyanobacteria, Cell free supernatant, Seed germination, Root length, fresh weight.**

I. INTRODUCTION

Cyanobacteria are oxygenic, photosynthetic, free living and nitrogen fixing microorganisms commonly found in fresh water, marine water and soil. Cyanobacteria are being used as biofertilizer for plants, as food for human consumption and for the extraction for various product such as vitamins, drug compound and human growth factors (Fay et al., 1983). The role of N2 fixing cyanobacteria in maintenance of rice fields has been well substantiated and documented all over the world. In India alone, the beneficial effect of cyanobacteria on yield of rice varieties have been demonstrated in a number of field location (Venkataraman et al., 1981). An additional benefit of cyanobacterial biofertilizers is their capacity to secrete bioactive substance such as auxin, gibberellins, cytokinins, vitamins, polypeptide, amino acid, which promote plant growth and development (Sergeeva et al,.2002). The effect of cyanobacterial inocula on the yield of crops in the presence of nitrogenous fertilizers has commonly been dscribed to the production of growth promoting substances by these organisms(Brown *et al.,* 1956; Kopteva, 1970; Tupik, 1973).A large number of researchers have found better growth and germination of seeds of many crop plants after treating them with algal cultures or their extracts. Pre-soaking of seeds in algal extracts has been shown to be beneficial for wheat and mung (Gupta et al,. 1970).Pre-soaked seeds in algal extracts resulted in 5 percent more grain production of mung and wheat compared to untreated seed (Tiwari et al., 1990).A number of studies have been carried out on the plant growth promoting nature of extracellular and intracellular products of cyanobacteria. Their beneficial effect on cereal and vegetable crops has been reported(Bongale*et al.,* 1980; Bongale et al,.1985). It is concluded that foliar spray of culture filtrates of cyanobacteria improved yields in one out of four crops in a year (Bongale et al,. 1990).

In present study a total of eight different algal strain were evaluated for seed germination experiments.

II. MATERIALS AND METHODS

Media and reagent

Sodium nitrate, dipotassium phosphate, magnesium sulphate, ammonium chloride, calcium chloride, ferric chloride, potassium dichromate, 1,5 diphenyl carbazide, methanol, hydrogen sulphate and ferrous ammonium sulphate were used of analytical grade. All this reagent and media were prepared in distilled water.

Collection of sample

From the present study,water sample were collected from different site i.e. various pond, lackes and riverin different localities from Daman, Vapi, Valsad, Gujarat-396001,India.

Isolation and Screening of Cyanobacteria

Water sample was inoculated in Erlenmeyer flask having algae culture medium (composition of medium in gm/lit; sodium nitrate 0.1 gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride, calcium chloride 0.005 gm and ferric chloride 0.0005 gm) and growth from the incubated flask were spreaded on algal culture plate at room temperature under continuous dark and sunlight period for 15-20 days as described by Patil*et.al.,* isolated colonies were observed in microscopic for morphological characterization.

Identification of cyanobacteria

Microscopic observation was done by spreading isolated culture on glass slide using forceps and needles. Culture was covered with glass cover slips and observed under low (10X) and high power (40X) objective lens of compound light microscope. Pure forms of cyanobacteria were identified on

the basis of morphological characteristics. (P. Khare*et al.*, 2014)

Seed germination experiment

The mung (*Vignaradiata*) and wheat (*Triticumaestivum*) seeds were collected from agriculture ,Vapi, Gujarat, India. Seeds were surface sterilized with 0.1 % $HgCl_2$ for 3 min.Ten viable seeds were tested for each algal aqueous extract. Seeds, without algal extract, served as control. Each petridish contain ten surface sterilized seeds were placed on filter paper and moistened with 10 ml of the aqueous extract of algae. Petri dishes containing seeds with 10 ml of distilled water served as a control. The growth parameters including germination percentage, root length and weight were recorded on the 3 days after incubating seed at 28^0C (Pitchai*et al.*, 2010).

III. EXPERIMENTAL OBSERVATIONS

Percentage germination (%)

The percentage germination was calculated by dividing number of seeds germinated to the total number of seeds used for soacking.

Root length

Root length of ten seedlings in each treatment and replication were measured in centimetre. The average root length were computed and expressed in centimetre.

Fresh weight (g) of seedlings

Ten seedlings from each treatment and replication were selected. Fresh weight of the seedlings was taken in gram. The average fresh weight were weighed and expressed in gram. Four most efficient isolates based on their observation of % germination, root length and fresh weight of seedlings were selected for further investigation.

IV. RESULTS AND DISCUSSION

Isolation and screening of Cyanobacteria culture

In present study various water samples from different regions of Vapi, Gujarat, India, were screened on algal plate and a total of 8 algal isolate were isolated in pure form. The pure forms of the isolate were maintained in 250 ml Erlenmeyer flask containg 100 ml algal culture medium at room temperature under continuous dark and sunlight period. Fig. 1 shows growth of all cyanobacterial isolates in algal culture medium. All the isolates were subjected for microscopic observation (Fig. 2) and identified on the basic of their cellular morphology as described by Khare*et al.*, (2014). The morphological characteristics and its identification is shown in table 1.

Fig. 1. Growth of cyanobacterial isolates in algal culture medium.

Table 1 Morphological characteristics of cyanobacterial isolates

Sr. No.	Cyanobacterial culture	Microscopic and Macroscopic observation	Morphotype
1	JH 1	long,vescicular, lobed, hyaline, filaments closely adpressed.	*Rivulariaspp*
2	JH 2	green,tightly packed and densely entangled in common mucilage, globose,filamentous,heterocystous.	*Nostocspp*
3	JH 3	Single, straight, filamentous, non-heterocystous,	*Oscillatoria spp.*
4	JH 4	Single-celled (solitary), crescent-shaped (bowed).being slightly widened in the middle and pointed on the ends.	*Closterium spp.*
5	JH 5	Unicellular cells, cells ellipsoidal to cylindrical.	*Gloeothece spp.*
6	JH 6	green, membranous, densely aggregated, cells barrel shaped and parallel (2.5-3µm), filamentous, heterocystous.	*Anabaena spp.*
7	JH 7	unicellular cells, loosely arranged in a group.	*Aphanocapsa spp.*
8	JH 8	cells spherical, 2-4 together, colourless sheath,	*Gloeocapsa spp.*

The morphological characteristics of the eight cyanobacterial species applied in this study was studied in 10x, 40x micrscope. Amongst them eight morphotypes were observed, three heterocytous and five non heterocytous cyanobacteria were identified.

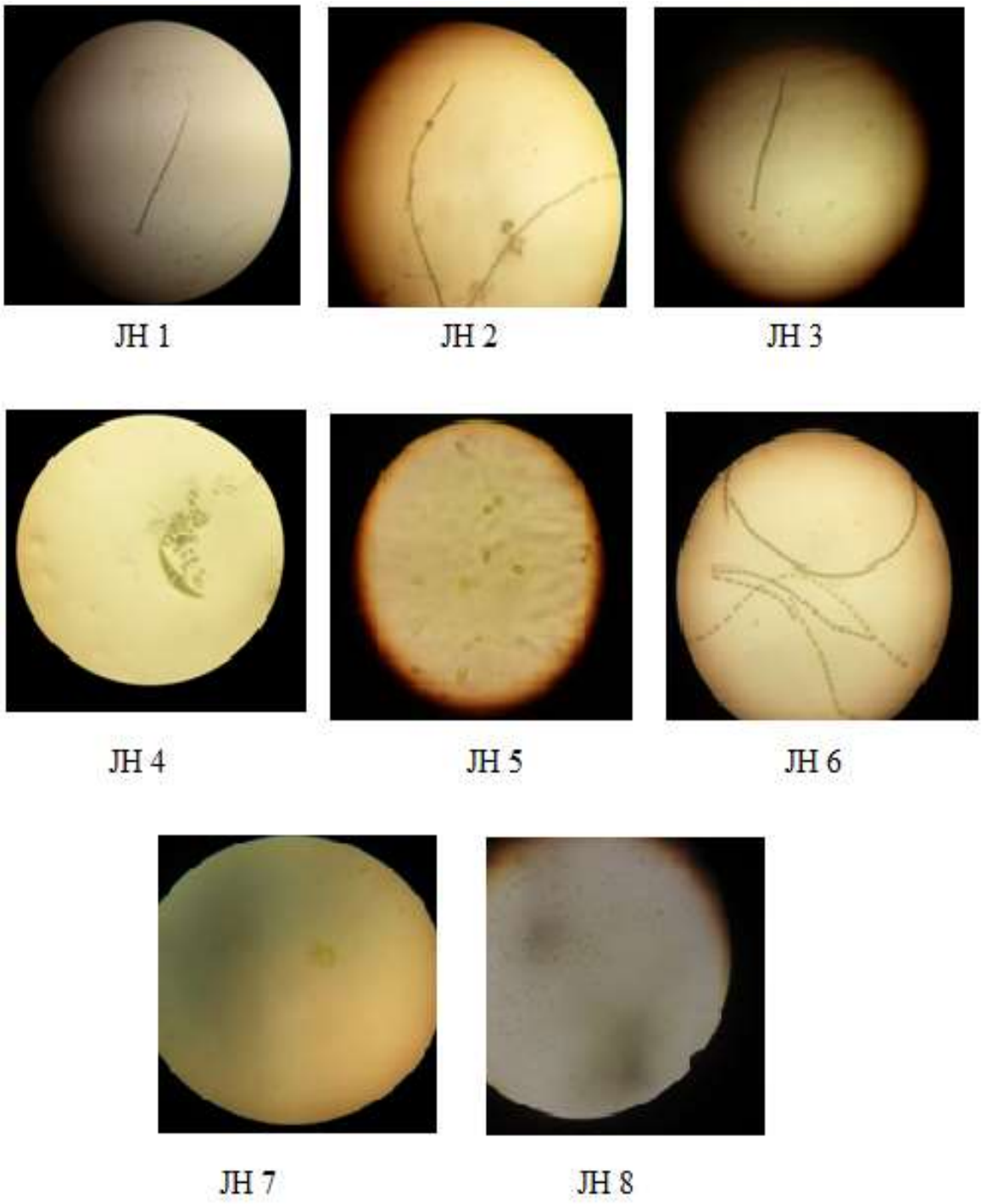

Fig.2. Microscopic observation of cyanobacterial isolates.

Effect of cyanobacterial extracellular extract on germination and growth of Mung andwheat seeds. The present study was undertaken to evaluate the ability of cyanobacteria on both germination and growth of mung and wheat seeds. Eight cyanobacterial isolates maintained in algal culture medium, isolates from the pond water were checked for germination rate, root length and fresh weight of seedlings for mung and wheat. The mean rates of germination observed after 24 hour, 48 hour and 72 hour for mung and wheat seeds using 21 day old and 28 day old cell free supernatant of cyanobacterial cultures applied separately are presented in Table 2(a), 2(b) and 3(a), 3(b) respectively. All the cultures tested showed a positive effect on seed germination of mung as well as wheat. The germination frequency was always more in presence of cell free supernatant of cyanobacterial cultures as compared to control, i.e. water.

Effect of cyanobacterial extracellular extract (21 day and 28 day) on germination and growth of Mung (Vignaradiata)

The extract of fifty percent algal strain showed 100% germination in case of 21 day cell free supernatant of cyanobacterial strains as shown in Table 2a and ninety percent of the strain showed 100% germination in case of 28 day cell free supernatant of cyanobacterial strains as shown in Table 2b on mung seeds.

Table 2a Effect of cell free supernatant (21 day) of cyanobacterial strains on mung seed germination

Sample	Seed germination (%)	Root Length (cm)	Fresh root weight (gm)
Control	90	3.03	0.041
JH 1	80	3.00	0.042
JH 2	100	3.39	0.051
JH 3	100	3.72	0.053
JH 4	100	3.91	0.067
JH 5	80	2.99	0.032
JH 6	90	3.27	0.048
JH 7	90	2.45	0.039
JH 8	100	3.17	0.044

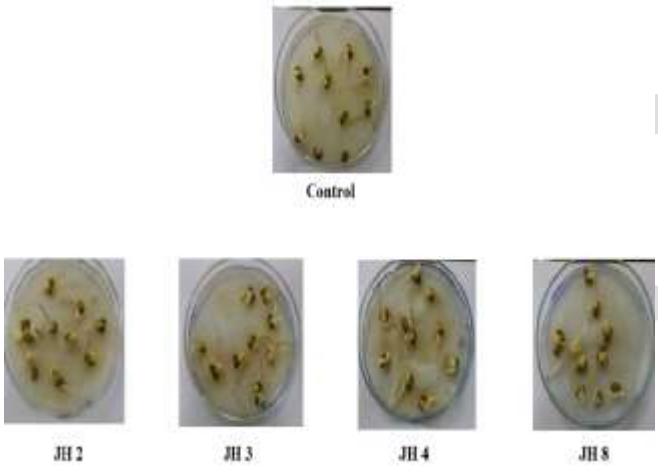

Fig. 3 Effect of cell free supernatant (28 day) of cyanobacterial strains on mung seed germination

Table 2b Effect of cell free supernatant (28 day) of cyanobacterial strains on wheat seed germination

Sample	Seed germination (%)	Root Length (cm)	Fresh root weight (gm)
Control	90	2.08	0.051
JH 1	100	2.84	0.066
JH 2	100	3.79	0.171
JH 3	100	3.99	0.054
JH 4	100	3.69	0.100
JH 5	90	3.00	0.044
JH 6	100	3.57	0.078
JH 7	100	2.98	0.059
JH 8	100	3.44	0.911

Highest seed germination observed in cyanobacterial cell free supernatant of isolates of JH 2, JH 3, JH 4 and JH 8 on mung seeds as compared to other (figure 3). The root length was more than 3.0 cm in case of JH 2, JH 3, JH 4, JH 5, JH 6 and JH 8 extract. The weight of root is more than 0.100 gm in case of JH 2, JH 4 and JH 8 extract.

Effect of cyanobacterial extracellular extract (21 day and 28 day) on germination and growth of wheat (Triticumaestivum)

Table 3a Effect of cell free supernatant (21 day) of cyanobacterial strains on wheat seed germination

Sample	Seed germination (%)	Root Length (cm)	Fresh root weight (gm)
Control	60	0.53	0.009
JH 1	80	0.67	0.013
JH 2	90	0.80	0.013
JH 3	90	0.87	0.017
JH 4	100	0.70	0.015
JH 5	60	0.38	0.007
JH 6	40	0.32	0.027
JH 7	50	0.96	0.009
JH 8	90	0.76	0.011

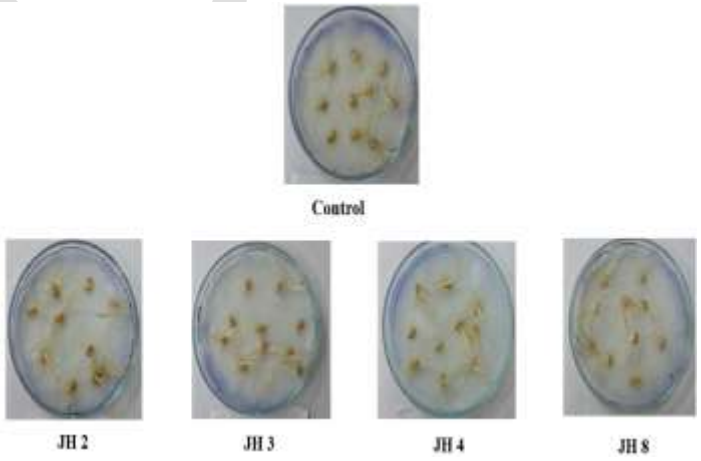

Fig. 4 Effect of cell free supernatant (28 day) of cyanobacterial strains on wheat seed germination

Table 3b Effect of cell free supernatant (28 day) of cyanobacterial strains on wheat seed germination

Sample	Seed germination (%)	Root Length (cm)	Fresh root weight (gm)
Control	70	2.0	0.009
JH 1	40	1.1	0.013
JH 2	90	2.2	0.013
JH 3	80	2.1	0.017
JH 4	100	2.7	0.015
JH 5	60	2.1	0.007
JH 6	60	2.2	0.027
JH 7	70	1.6	0.009
JH 8	80	2.5	0.011

The extract of JH 4 showed 100% germination in both case. However the 28th day extract of four isolate JH 2, JH 3, JH 4 and JH 8 showed more than 80% seed germination. The root length of germinated seeds was also higher than the 2.0 cm when treated with extract of JH 2, JH 3, JH 4 and JH 8. The effect of cell free supernatant of cyanobacterial cultures on fresh weight of seedlings had showed a variable response and it was interesting to note that cell free supernatant of 28 day old cultures had comparatively better positive effect on fresh weight of wheat seedlings and mung seedlings.Brahmbhatt and Kalasariya., (2015) reported that when plate culture of alfalfa was treated with extract of *Spirogyra* sp. and*Oscillatoria*sp ,the germination of seeds was faster as compared to seeds soaked in distilledwater as control. Similar result had been shown by Patil*et al.,* (2015). The PGPR production profile and seed germination experiment shows that cyanobacterial isolate JH2, JH3, JH4 and JH8 are potential isolates for PGPR response.

V. SUMMARY AND CONCLUSION

The objective of the present was to isolate algal strain from various water samples in the region of Vapi, Gujarat-396195, and India. A total of 8 algal strains were isolated and identified on the basis of their macroscopic and microscopic characteristics. All the isolated algal culture was further study. All the 8 algal isolate was grown in algal culture medium to produce various plant growths promoting regulators.The 21st day extract of JH1, JH2, JH3 and JH 8 shows 100% mung seed germination as compare to other isolate. The 28th day extract of isolate JH 2, JH 3, JH 4 and JH 8 showed more than 80% seed germination and root length greater than 2.0 cm of wheat seeds.

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